# REDUCTION IN LIGHT REFLECTANCE OF LEAVES OF *ENCELIA DENSIFOLIA* (ASTERACEAE) BY TRICHOME WETTING

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## ABSTRACT

*Encelia densifolia* is a desert shrub endemic to a single mountain range in the central western portion of the Baja California peninsula. When dry, its pubescent leaves reflect about as much light as other pubescent *Encelia* species, such as *E. actoni* and *E. palmeri,* although not as much as the densely tomentose *E. farinosa.* When the leaves are wet, their trichomes absorb water, and reflectively is decreased to a level comparable to nearly glabrous *Encelia* species, such as *E. californica* and *E. frutescens.* This response to wetting is likely to be advantageous to the plants; they inhabit a region of summer fogs, where light intensity and air temperature are reduced and relative humidity is increased by the same conditions that reduce leaf reflectance.

## RESUMEN

*Encelia densifolia* es un arbusto del desierto, endémico a una sola sierra en la comarca occidental del centro de la peninsula de Baja California. Cuando secas, sus hojas pubescentes reflejan casi tanto como otras especies pubescentes de *Encelia,* tal como *E. actoni* y *E. palmeri,* aunque no tanto como la muy tomentosa *E. farinosa.* Cuando las hojas son mojadas, sus tricomas absorben el agua, y la reflectividad disminue a un nivel análogo a especies pelonas, tal como *E. californica* y *E. frutescens.* Esta respuesta a lo mojando es probable que será ventajosa a las plantas. Habitan una comarca de nieblas estivales, dónde la intensidad de la luz y la temperatura del aire se reducen y la humedad relativa se aumenta por las mismas condiciónes que reducen la reflectividad de las hojas.

A number of studies have been conducted in recent years concerning the adaptive nature of leaf pubescence. The genus *Encelia* has been one of the most intensely investigated, particularly *E. farinosa* and the role the pubescence plays in the adaptation of this species to its desert climate (Ehleringer and Clark 1988, and references cited therein). Ehleringer and others have shown that leaf hairs are important to the success of the plants in their hot, dry habitat, because pubescence increases reflection of solar radiation, lowering leaf temperatures and rates of water loss (Ehleringer and Clark 1988).

A newly described species of *Encelia, E. densifolia* Clark & Kyhos, is similar to other species of the genus in that a dense pubescence of multicellular uniseriate hairs covers both surfaces of the leaves

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(Clark et al. 1988). Although the trichomes form a continuous cover over the surface of the leaves, we have found that the leaves are not as reflective as those of other species such as *E. palmeri* and *E. farinosa* (Ehleringer and Clark 1988). Moreover, unlike those of *E. farinosa*, the trichomes of *E. densifolia* absorb water easily and in doing so lose most of their reflective capacities. *Encelia densifolia* is endemic to canyons in the Picachos de Santa Clara, Baja California Sur, México, where fog is common in the summer, and Clark et al. (1988) suggested that the loss of reflectivity of the leaves could be an adaptation to increase light capture on foggy days.

Our purpose in this study was to compare the differences in leaf light reflectance between dry and wet leaves of *Encelia densifolia*, to compare these results with those of the sympatric and well-studied species *E. farinosa*, and finally to determine what physical features of the trichomes of these two species account for the differences.

## Materials and Methods

All studies were conducted with plants grown from seed and maintained outdoors at California State Polytechnic University, Pomona (methods follow Kyhos et al. 1981). All the seeds were obtained from plants growing in native habitats in México, in the state of Baja California Sur (vouchers at CSPU): *Encelia densifolia*—Picachos de Santa Clara, 13.6 mi NW of San Ignacio–Abreojos road at a point 24.7 mi NE of Punta Abreojos, 24 Mar 1981, accession 184, *Clark 585*. *Encelia farinosa*—Pemex station on Mex Hwy 1 at San Ignacio, 23 Mar 1981, accession 183; S of Bahía Concepción at Microondas Rosarito, 25 Mar 1981, accession 186; S end of Bahía Concepción, near the beach, 27 Mar 1981, accession 190.

We measured leaf reflectance by placing the individual leaves (adaxial side exposed) in the sample slot of the integrating sphere of a Shimadzu/Bausch & Lomb Spectronic 210 U.V. recording reflectance spectrophotometer. Reflectances were measured at the violet (425 nm) and red (670 nm) peaks of the photosynthetic action spectrum (Jensen and Salisbury 1984, p. 81). Magnesium oxide powder (Wako Pure Chemical Industries, supplied with the integrating sphere) was used as a reference. To insure accuracy of the reference, two blanks were prepared and calibrated against each other; if either deviated from the range of 99%–101% compared to the other, both were discarded, and new references were prepared.

Ten ontogenetically mature leaves were selected at random from each of ten plants of *Encelia densifolia* during the period from 7 to 13 October, 1986, and six leaves were used from each of twelve plants of *E. densifolia* on 2 February 1987. During the fall, individual leaves of *E. densifolia* were sometimes not large enough to cover the opening in the integrating sphere (18 mm diameter); in those cases, two leaves were overlapped, giving sample sizes less than 10. For comparison, ten leaves from each of three plants of *E. farinosa* were taken in 2 February. For each leaf, reflectance was measured, the leaf was wetted with deionized water, excess water was allowed to drain, and reflectance was measured again. The same wetting procedure was used to generate five reflectance spectrograms over the range of 400–700 nm.

We tested for the presence of cuticle on the trichomes of both species with Sudan Black B (a histochemical stain for lipids) used on freehand sections and epidermal peels of fresh leaves.

We estimated the rate of water absorption by the trichomes by dropping two drops of water in separate locations on each of four leaves of both species, and measuring the elapsed time until each drop was absorbed. To assess the effect of cuticle removal, we performed three experiments. In each, leaves were dipped in 95% acetone for varying times, allowed to air-dry, and the absorption rate test was performed as described above. In the first experiment, four leaves of each species were dipped for 1 sec while still attached to the plants, and allowed to recover for 86.4 ksec (1 day). *Encelia densifolia* leaves were not used in the other two experiments, since water absorption in the first experiment was nearly instantaneous. In the second experiment, four leaves were removed from the plant, dipped in acetone for 1 sec, and measured immediately upon drying. In the third experiment, five leaves were removed from the plant, dipped for 300 sec, and measured immediately upon drying. In all cases, timing of the absorption of an individual water drop was discontinued if it had not been absorbed after 60 sec.

Fog simulation was accomplished with a Fogg-It "Waterfog" Nozzle, rated at one-half gallon per minute (31.5 ml/sec). We sprayed the fine mist upwind from the plants to mimic actual fog conditions. Effects on the leaves were assessed visually.

All statistical analyses were performed with the MINITAB statistical package on the California State University Central Cyber System computer.

## Results

We found significant differences in reflectance between wet and dry leaves of *Encelia densifolia*. Over the entire spectrum from 400 nm to 700 nm, the reflectance of wet leaves was 50–60% of that of dry leaves (Fig. 1), whereas in *E. farinosa* there was no appreciable change (data not shown). At both 425 nm and 670 nm, wet *E. densifolia* leaves were significantly less reflective than dry leaves, for both the fall and winter samples. In contrast, there were no significant differences between wet and dry *E. farinosa* leaves at both wavelengths (Fig. 2). These results also held for each individual plant of both species (two-sample t-test, $p < 0.05$).

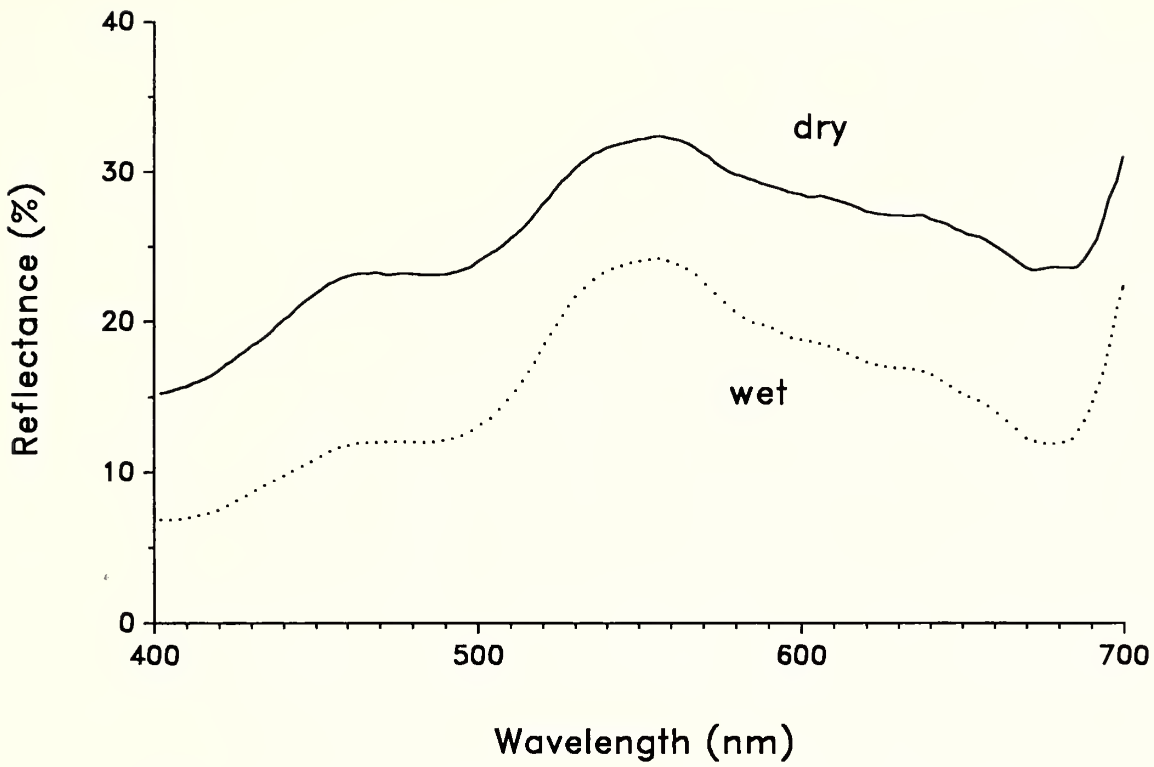


FIG. 1. Reflectance spectra of a representative leaf of *E. densifolia,* before and after wetting of the trichomes.

Although it was not our intent to assess seasonal changes in reflectivity, because the plants are freely watered in cultivation, we did find differences between the October and February samples. At 425 nm, dry leaves were significantly more reflective in October (two sample t-test, $p < 0.05$), but there was no significant difference between wet leaves. At 670 nm, both wet and dry leaves were significantly more reflective in February ($p < 0.01$).

In the wetting experiment, most areas on an untreated leaf of *E. densifolia* were saturated by water in under 1 sec. With untreated *E. farinosa* leaves, saturation generally had not occurred at 60 sec, when measurement was discontinued, and on one leaf neither water droplet had been absorbed after more than 2 ksec. Even when such long wetting times are recorded as 60 sec, there is a significant difference in wettability between the two species (Mann-Whitney U-test, $p < 0.0001$).

Although cuticle of the epidermal cells of both species was stained by Sudan Black B, the trichomes of both species remained generally unstained, with only a very few of the basal cells staining. Wettability decreased in acetone-dipped leaves, however, to the extent that *E. farinosa* leaves treated for five minutes with acetone were, with the exception of a single leaf, just as absorbent as leaves of *E. densifolia* (Fig. 3).

As expected, simulated fog caused an immediate reduction in leaf reflectance of *Encelia densifolia*; the fine water droplets were quickly absorbed by the trichomes. With *E. farinosa,* on the other hand, the

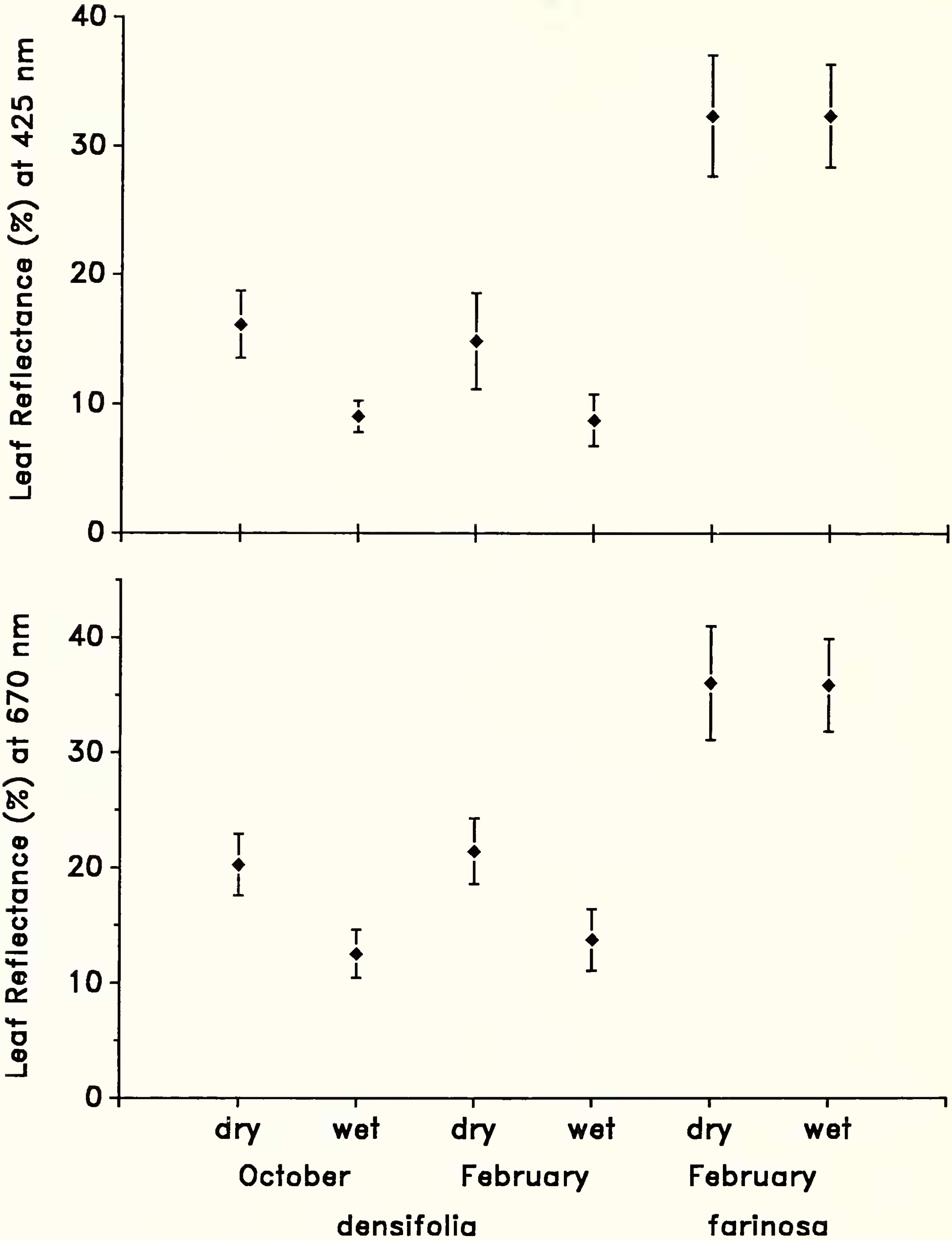


FIG. 2. Reflectance of wet and dry leaves of *E. densifolia* and *E. farinosa*. Means are marked by diamonds, and error bars represent ±1 SD. Means were significantly different (two-sample t-test, $p < 0.001$) for wet and dry *E. densifolia* leaves in both seasons.

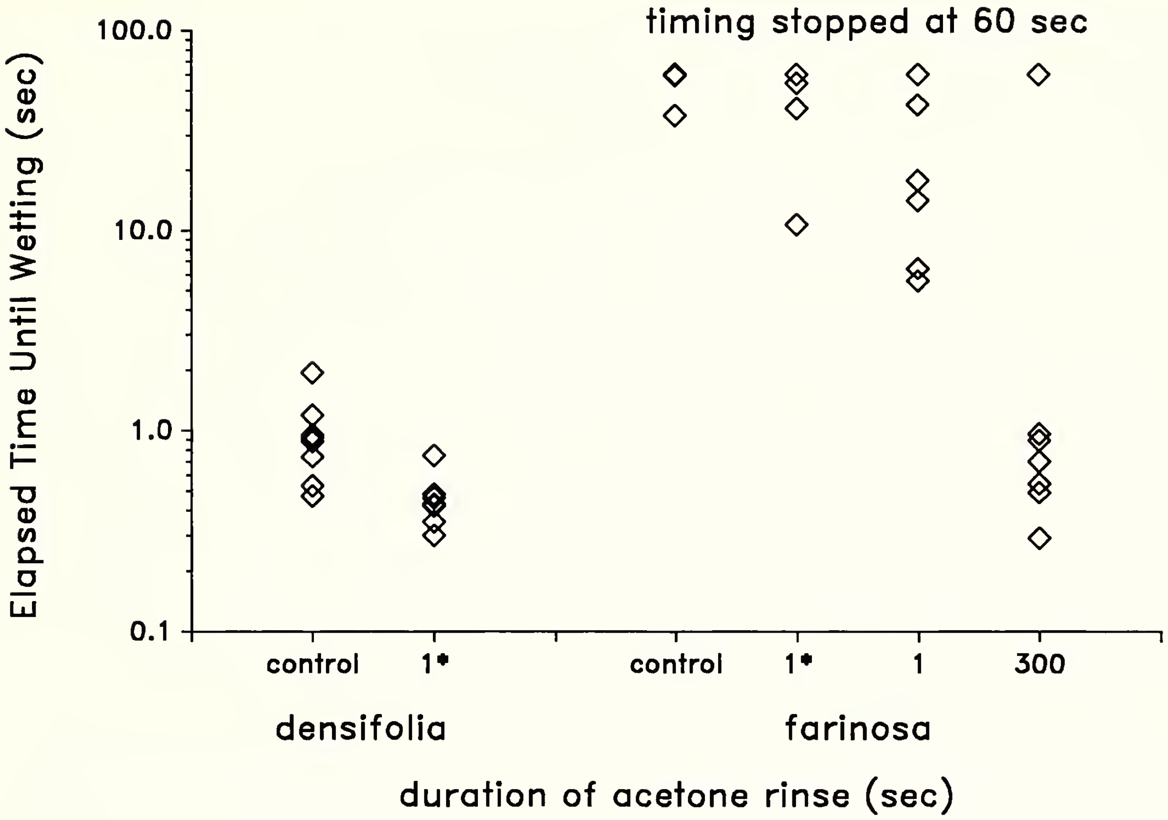


FIG. 3. Univariate scatterplot of elapsed time until a drop of water was absorbed by the leaf trichomes of *E. densifolia* and *E. farinosa* (see text for explanation).

water droplets beaded up on the surface of the trichome layer, eventually coalescing and running off the leaf.

## DISCUSSION

When dry, the leaves of *Encelia densifolia* are as reflective as other pubescent-leaved *Encelia* species, such as *E. actoni* and *E. palmeri*. When they are wet, their reflectance decreases to a level comparable to nearly glabrous species, such as *E. californica* and *E. frutescens* (Ehleringer and Clark 1988). The well-studied *E. farinosa*, which is sympatric with *E. densifolia*, shows no changes in reflectance between dry and wet leaves, and in fact the leaf trichomes are not wettable.

Although histochemical staining showed no cuticle layer on the trichomes of either species, the hydrophobic nature of *E. farinosa* trichomes was sharply reduced by an acetone rinse. This is consistent with the removal of cutin or some other lipophilic substance.

We believe these differences can be accounted for by the unusual environmental conditions in the habitat of *E. densifolia*. The region is characterized by frequent fog from the Pacific Ocean during the summer, especially in May, June, and July, the driest months of the year (Wiggins 1980). During fog conditions the leaf trichomes become wet and the leaves lose most of their reflectivity. This decreased reflectivity comes at a time when it is most beneficial (light intensity is reduced by the fog) and least harmful (air temperatures are reduced and relative humidity is very high).

In the same environment, the leaves of *E. farinosa* maintain their reflectance regardless of atmospheric conditions. That *E. farinosa* and pubescent species of other genera thrive in the area is an indication that the adaptation exhibited by *E. densifolia* is not a general requirement. On the other hand, *E. farinosa* is the most widely distributed species in the genus (Shreve and Wiggins 1964), while *E. densifolia* is not found outside of the fog zone. Although there are undoubtedly other factors involved in this restriction, we believe that the unique adaptation of *E. densifolia* helps it survive as a relict in a generally unsuitable region, rather than giving it an advantage over other desert shrub species.

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